

claim amendments and additions are fully supported by the specification as described below and contain no new matter. Claims 1-25 and 28-95 are now currently pending in the application.

To simplify prosecution of related cases, Applicants will abandon applications 09/297,418 (with priority to PCT/US97/20441) and 09/398,966 (with priority to 08/740,682). Although certain claims may have been narrowed in scope, it is the intent of the Applicants to broaden the overall scope of the claims as a whole in order to better encompass the full scope and breadth of the invention. Polypeptides and seed with elevated essential amino acids, primarily lysine but also histadine, are disclosed in the '966 application. More advanced embodiments, such as seed and polypeptides with a stabilizing disulfide bond and greater levels and better balances of essential amino acids, are disclosed in the '418 application. Applicants submit that many of the claims are fully supported by the earlier filed applications and intend that the claims draw priority to the earliest filed application with support for the claim. In some cases this has been specifically noted by Applicants. However, Applicants' failure to refer to an earlier filed application in support of the claim does not imply that Applicants believe that such claims were not supported by the earlier filed application. Pages and line numbers are to the '689 application unless otherwise noted.

The principal aspect of this invention is the genetic modification of protein to increase the essential amino acid content of a plant seed. Claims 1-8 have been amended to claim a plant seed because Applicants view this as the most likely commercial embodiment of the invention.

Claims 1-9 have been amended to replace the terms CI-2 like with the term CI-2 derived. The term "CI-2 derived" is defined on page 8, lines 13-15. Most of the Sequence ID's in the '689 and related applications are examples of CI-2 derived polypeptides.

Claim 1 and also claims 9, 10, 19, 21, 28, 29, 30, 31 and 32 have been amended to delete the term isolated. The polypeptides of the present invention have been modified by man to include non-native amino acids and, therefore, the claims as amended are not drawn to a product existing in nature.

Claims 2 has been amended to specify the protein modification is post-translational. Examples of post-translational protein modifications are provided on page 14 line 27 through page 16 line 5.

Claim 3 has been amended to claim that which Applicants believe to be the best mode of the invention, which is the modified polypeptide with a stabilizing disulfide bond that protects the polypeptide from degradation. A non-native disulfide bond was first disclosed on page 65, bottom paragraph, of application PCT/US97/20441, and is also disclosed throughout the Examples in the '689 application. Sequence ID No. 20 is one embodiment of this aspect of the invention.

Claim 7 clarifies that the activity of the polypeptide, if any, is that of a protease inhibitor.

Claim 8, 9 and 11 add isoleucine to the list of essential amino acids claimed. Isoleucine is defined as an essential amino acid on page 5, lines 5 to 8.

Claim 9 claims a CI-2 derived polypeptide of at least 50 amino acids. Support for a polypeptide fragment is provided on page 11 lines 7-28 and on page 26 line 29 to page 27 line 2. A substituted CI-2 like polypeptide with about 55% to about 90% essential amino acid content is taught on page 42, lines 25-26 and page 44, lines 27-30. A CI-2 derived polypeptide with more than 32 non-native essential amino acids is taught on page 41, lines 30. Polypeptides with various percentages of essential amino acids are disclosed throughout the specification, claims and in the polypeptides provided as Sequence ID's. In particular, over its 67 amino acids, Sequence ID. No. 20 contains 23.9 mole % lysine and 9.0% methionine. Applicants have also demonstrated an additional

embodiment of the invention with 67 amino acids and 6.0 mole % tryptophan, 16.4 mole % threonine and 11.9 mole % isoleucine, and can provide documentation of such embodiment by declaration if necessary. For reference, out of its 66 amino acids, truncated wild type CI-2, represented by Sequence ID. No. 4, contains 9.1 mole % lysine, 3.0 mole % methionine, 4.6 mole % threonine, 1.5 mole % tryptophan and 9.1 mole % isoleucine.

Claims 18 and 22 have been amended to correct typographical errors.

Claims 19, 21, 28, 30 and 50 have been amended to place the claims in proper Markush group format.

Claims 26 and 27 have been deleted because of the complexity in the way the claims were written. Such claims have been replaced by many of the newly added claims, including claims 61-77.

Claims 47, 49 and 50 were included in Group II (the non-elected group) of the restriction requirement on the basis that such claims are drawn to polynucleotides. However, Applicants have traversed the restriction requirement with respect to these claims, as provided in the response to the restriction requirement filed of even date herewith. This traversal is based on the the fact that such claims are drawn to polypeptides, which is the subject matter of the elected restriction group. In the event the Examiner elects to examine these claims, Applicants have amended these claims to more clearly define the invention. Claim 47 is now drawn to a method incorporating the seed of claim 1. Claim 49 is drawn to a method utilizing the seed of claim 1 which also comprises a non-native disulfide bond. Claim 50 is drawn to a method of altering a CI-2 homologue.

New claims 52, 53, 55, 56 and 91 have been added to direct claims to food and feed comprising the polypeptide, as the seed comprising the polypeptide will most likely

be used for food and feed. Use in food and feed is disclosed throughout the specification, in particular, in the Field of the Invention on page 1.

New claims 54, 56 and 57 are drawn to high lysine embodiments of the invention. The '966 application discloses various high lysine embodiments of the invention, including specific embodiments with 21.5% and 25.3% lysine (see page 8 of the '966 application).

New claims 59 and 60 are drawn to CI-2 derived polypeptides of at least 50 amino acids that have certain levels of methionine and threonine, respectively. These claims are supported as described for claim 9 above.

New claims 61-77 are directed to a CI-2 derived polypeptide that has been modified to increase the amount of essential amino acids in the polypeptide. Sequence ID No. 20 contains a non-native disulfide bond and 25 non-native essential amino acids. The polypeptide as claimed in claim 13 contemplates 34 locations for substitution of native amino acids for non-native essential amino acids. Page 44 lines 24-26 disclose various numbers of amino acids that may be substituted with non-native essential amino acids. Support for a polypeptide fragment with 23 amino acids is provided on page 11 lines 7-9 and on page 26 lines 29 to page 27 line 2.

New claim 62 uses the terms conservatively modified and conservatively substituted variant. These terms are defined on page 9, line 17 to page 10 line 30 and on page 17, lines 3 – 25.

New claim 65 uses the term amino terminal extension, which is disclosed on page 43, lines 15-22, page 67 lines 15-31 and by Seq. ID 12. Sequence ID's in the earlier applications also contain amino terminal extensions. These amino terminal extensions contain non-native essential amino acids and/or a start methionine, as claimed in new claims 66 and 67 and described in the specification.

New claims 68-71 are drawn to embodiments containing a non-native disulfide bond as disclosed on page 65, bottom paragraph, of application PCT/US97/20441, and also throughout the Examples in the '689 application.

New claims 72-77 are directed to a CI-2 derived polypeptide that has been modified in a way that will increase the amount of essential amino acids in the polypeptide. Support for a polypeptide fragment with 23 amino acids is provided on page 11 lines 7-9 and on page 26 lines 29 to page 27 line 2. Support for an embodiment of such invention containing enhanced lysine levels, as described in new claims 73, 75 and 77, is found in the Sequence ID's of the '966 application. Page 44 lines 16-26 disclose various numbers of amino acids that may be substituted with non-native essential amino acids, including specific support for the numbers 4, 7, 11 and 34.

New claim 78 is directed to variants of the modified polypeptides of the present invention by measuring similarity in relation to the truncated wild type CI-2 (Sequence ID No. 4). Support for a polypeptide with 64% identity is provided on page 12, lines 1-3 and page 16, lines 14-18. This invention was first taught in the PCT/US97/20441 application, with Sequence ID. No. 20 of the '689 application showing an actual embodiment of this invention containing the disulfide bond. Sequence ID. No. 20 shows 65% identity with the full length of Sequence ID. No. 4. Applicants have also demonstrated an additional embodiment of the invention with 64% identity with the full length of Sequence ID No. 4, and can provide documentation of such embodiment by declaration if necessary.

New claims 79 - 84 are directed to variants of the embodiments of the invention exemplified by Sequence ID. Nos. 6, 8 and 10 respectively, which were first taught in the PCT/US97/20441 application. Support for polypeptides with 57%, 60%, 67% and 74% identity is provided on page 12, lines 1-3 and page 16, lines 14-18. Further support is provided by the specification's teaching regarding the number of substitutions that can be made, as on page 44, since this can be easily converted into percent identity. For example, a sequence with 26 amino acid substitutions out of the 66 amino acids

represented by positions 18-83 in Sequence ID Nos. 6, 8 or 10 would be the equivalent of saying the sequence has 60.6% identity. The non-native disulfide bond described in claims 79-84 and in claims 86 and 87 is taught on page 65, bottom paragraph, of application PCT/US97/20441, and also throughout the Examples in the '689 application.

New claims 87 – 92 are directed to modifications made to polypeptides homologous to CI-2. As taught by Applicants on page 8, lines 20-32, polypeptides homologous to CI-2 may be modified to create the polypeptides of the present invention. The term homologue is defined by the text on page 8, lines 16 – 32, and by the numerous examples of known homologues that are provided, including those in Sequence ID. Nos. 24, 26, 28, 30 and 32. Figure 2 of the '689 application provides a sequence alignment of many of the homologues known as of the filing date of the '689 application.

New claim 93 is drawn to a method for increasing the essential amino acid content in a polypeptide, which was achieved in the sequence exemplified in Sequence ID No. 20. New claims 94 and 95 are limited to various embodiments of this invention.

All claims as amended by this preliminary amendment are fully supported by the specification as filed. Applicants respectfully request that the Examiner consider these claims as now amended.

Should the Examiner have further questions or comments with respect to examination of this case, it is respectfully requested that the Examiner telephone the undersigned so that further examination of this application can be expedited.

Respectfully submitted,

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We claim:

1. (once amended) A seed comprising a CI-2 derived polypeptide, which CI-2 derived polypeptide is modified to comprise a non-native essential amino acid residue in more than about 11% to less than about 80% of the amino acid residues.
2. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide further comprises post-translational protein modification.
3. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide further comprises from one to five disulfide bonds.
4. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide exhibits a free energy of unfolding of more than about 3.5 to less than about 15 Kilocalories per mole.
5. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide is proteolytically stable, as demonstrated by detection of the intact polypeptide based upon detection by SDS-PAGE analysis, following a 30 minute incubation at 37°C in 100mM Tris-HCl, 50mMNaCl, 1mM CaCl_2 , pH 8, with a 10:1 (weight to weight ratio) of polypeptide:protease, with the protease being either chymotrypsin or trypsin.
6. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide further comprises an amino-terminal extension.
7. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide exhibits a modified protease inhibitory activity.
8. (once amended) The seed of Claim 1 wherein the non-native essential amino acid residues comprise isoleucine, lysine, threonine, tryptophan, methionine or combinations thereof.
9. (once amended) A plant CI-2 derived polypeptide of greater than 50 amino acids in length and altered to have the following composition: 15-35 mole % lysine, 8-15 mole % methionine, 13-25 mole % threonine, 6-12 mole % tryptophan, 12-20 mole % isoleucine or combinations thereof.
10. (once amended) A polypeptide comprising Seq. ID. No. 2, or truncated versions thereof, modified to contain 7 or more non-native essential amino acid residues at

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positions corresponding to the positions in Sequence ID. No. 2 selected from 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81, 82 or combinations thereof.

11. (once amended) The polypeptide of Claim 10 wherein the essential amino acid residues comprise isoleucine, lysine, threonine, tryptophan, methionine, or combinations or conservative substitutions thereof.
12. (no change) The polypeptide of Claim 10 wherein the protein exhibits reduced inhibitory activity against chymotrypsin, subtilisin or elastase.
13. (no change) The protein of claim 10 wherein the polypeptide comprises one or more of the following modifications: V32T; E45T; D64T; D74T; or A77T.
14. (no change) The protein of claim 10 further comprising one of the following modifications: [T22C, V82C], [E23C, R81C] or [V53C, V70C].
15. (no change) The polypeptide of Claim 10 further comprising an amino-terminal extension.
16. (no change) The protein according to claim 15 wherein the amino terminal extension comprises a nutritionally-enhancing polypeptide.
17. (once amended) The polypeptide of Claim 15 wherein the amino-terminal extension is a start signal, a transit sequence, a transit peptide, a signal peptide, a fusion protein, a cleavable peptide, a CI-2 like polypeptide or an uncleaved peptide.
18. (once amended) The polypeptide of Claim 15 wherein the polypeptide comprises at least 1 to about 18 additional residues corresponding to amino acid residues 1 to 18 of Seq. ID No. 2 or 12.
19. (once amended) A CI-2 derived polypeptide comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:
H18A, I, L, V or M; N19K or T; L20M I, or V; E23T or K ; S31T or K; E34K or T; V38M I, or L; L40M I, or V; Q41K or T; Q47K or T; I49M I, L, or V; I56K or

T; M59G; R62K or T; I63M, L, or V; R65K or T; R67K or T; F69W; L73K or T; A75K or T; Q78K or T; V79T or K; and R81K or T.

20. (no change) The polypeptide according to claim 19 wherein the modifications comprise one or more of the following modifications: [E23C and R81C] or [T22C and V82C] or [V53C and V70C].

21. (once amended) A CI-2 derived polypeptide comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:

H18A or M; N19K; L20M; T22C; E23T or C; S31T; E34K; V38M; L40M; Q41K; Q47K; I49M; I56K; M59G; R62K; I63M; R65K; R67K; F69W; L73K; A75K; Q78K; V79T; R81K or C; and V82C.

22. (once amended) The CI-2 derived polypeptide of claim 21, further comprising substituting a tryptophan at position 61.

23. (no change) The polypeptide of claim 22, further comprising threonine at one or more of positions 32, 45, 53, 64 or 70.

24. (no change) The polypeptide according to claim 22 wherein the modifications comprise one or more of the following modifications: [E23C and R81C] or [T22C and V82C] or [V53C and V70C].

25. (no change) The polypeptide according to claim 22 further comprising an insert in the active site loop region that is enriched in essential amino acids for the purpose of nutritional enhancement.

26. (Deleted)

27. (Deleted)

28. (once amended) A CI-2 derived polypeptide with three or more non-native essential amino acids at positions selected from the group consisting of 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 32, 34, 38, 40, 41, 45, 47, 49, 56, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 73, 74, 75, 76, 77, 78, 79, 81 and 82; and including conservatively modified and conservatively substituted variants thereof; and excluding V, P, W, S, E and R at position 56; S, K, R, P, E, V, Y, W, and A at

- position 58; R, Y, P, W, E, V, S, K, and A at position 59; Q, S, T, I, P, and K at position 60; V, E, R, P, and W at position 61 and E, Q, N, V, F, and Y position 62.
29. (once amended) A polypeptide comprising Seq. ID No. 6, 8, 10, 12, 14, 16, 18, 20 or conservatively modified or conservatively substituted variants thereof.
30. (once amended) A polypeptide comprising at least 23 contiguous amino acids of SEQ. ID Nos. 6, 8, 10, 12, 14, 16, 18 or 20.
31. (once amended) A polypeptide comprising at least 23 contiguous amino acids with more than 79% sequence identity, to the polypeptide of Seq. ID No. 20, wherein the % sequence identity is based on the 23 contiguous amino acids sequence and is determined by GAP analysis using Gap Weight of 12 and Length Weight of 4.
32. (once amended) A CI-2 derived polypeptide that is immunologically reactive with antibodies against the protein of Seq. ID No. 20 and not SEQ ID No. 2.
33. (no change, non-elected claim) An isolated nucleic acid comprising:
- (a) a polynucleotide encoding the protein of claim 1;
 - (b) a polynucleotide that encodes a polypeptide of SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, or 20;
 - (c) a polynucleotide amplified from a plant nucleic acid library using the primers of SEQ ID NOS: 21 and 22;
 - (d) a polynucleotide comprising at least 20 contiguous bases of SEQ ID NOS: 5, 7, 9, 11, 13, 15, 17 or 19;
 - (e) a polynucleotide encoding a plant CI-2-derived polypeptide having 15% more essential amino acids than SEQ ID NO 2;
 - (f) a polynucleotide having at least 73% sequence identity to SEQ ID NO: 19, wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0;
 - (g) a polynucleotide comprising at least 25 nucleotides in length which hybridizes under low stringency conditions to a polynucleotide having the sequence set forth in SEQ ID NOS: 19, wherein the conditions include

hybridization with a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37°C for 24 hours and a wash in 2X SSC at 50°C, 3x for 15 minutes;

- (h) a polynucleotide comprising the sequence set forth in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17 or 19;
- (i) conservatively modified variants of SEQ ID NO : 5, 7, 9, 11, 13, 15, 17 or 19; or
- (j) a polynucleotide complementary to a polynucleotide of (a) through (i).

34. (no change, non-elected claim) The isolated nucleic acid of claim 33 wherein the polynucleotide is a plant polynucleotide.

35. (no change, non-elected claim) A vector comprising at least one nucleic acid of claim 33.

36. (no change, non-elected claim) An expression cassette comprising at least one nucleic acid of claim 33 operably linked to a promoter, wherein the nucleic acid is in sense or antisense orientation.

37. (no change, non-elected claim) A host cell into which is introduced at least one expression cassette of claim 36.

38. (no change, non-elected claim) The host cell of claim 37 that is a plant cell.

39. (no change, non-elected claim) A transgenic plant comprising at least one expression cassette of claim 36.

40. (no change, non-elected claim) The transgenic plant of claim 39, wherein the plant is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.

41. (no change, non-elected claim) A seed from the transgenic plant of claim 40.

42. (no change, non-elected claim) The seed of claim 41, wherein the seed is from corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley lupin or millet.

43. (no change, non-elected claim) A ribonucleic acid sequence encoding a polypeptide of claim 10.

44. (once amended, non-elected claim) A method for increasing the essential amino acid content in a plant, comprising:
- (a) stably transforming a plant cell with the polynucleotide encoding the polypeptide of claim 10, operably linked to a promoter, wherein the polynucleotide is in sense orientation;
 - (b) growing the plant cell under plant growing conditions to produce a regenerated plant; and
 - (c) growing plants for a time sufficient to express the polypeptide encoded by the polynucleotide of (a) in the plant.
45. (no change, non-elected claim) The method of claim 44, wherein the plant cell is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.
46. (once amended, non-elected claim) A method of increasing expression levels of a polypeptide in a transgenic plant cell comprising:
- engineering a nucleotide sequence encoding the polypeptide to contain a non-native disulfide bond to increase the in-vitro proteolytic or thermodynamic stability of the polypeptide; introducing at least 1 copy of the polypeptide into the transgenic plant cell; and growing the transgenic plant cell for a time sufficient to express the polypeptide.
47. (once amended, non-elected claim but w/ request to examine) A method of increasing the nutritional value of a feed or food source comprising producing the seed of Claim 1 and incorporating such seed into the feed or food source.
48. (deleted)
49. (once amended, non-elected claim but w/ request to examine) The method of Claim 47 wherein the seed of Claim 1 further comprises one or more non-native disulfide bonds.
50. (once amended, non-elected claim but w/ request to examine) A method of increasing the nutritional value of a Cl-2 homologue to enhance its nutritional value by altering two or more of the amino acid residues at positions selected

from the group consisting of: 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81 and 82 to have an essential amino acid, wherein the positions are determined based on homology to the amino acid positions in Sequence ID No. 2.

51. (deleted) An isolated nucleic acid comprising:

- (a) a polynucleotide of Seq ID Nos 23, 25, 27, 29 and 31;
- (b) a polynucleotide that encodes a polypeptide of SEQ ID NOS: 24, 26, 28, 30, 32;
- (c) a polynucleotide amplified from a Zea mays nucleic acid library using the primers of SEQ ID NOS: 21 and 22;
- (d) a polynucleotide comprising at least 20 contiguous bases of SEQ ID NOS: 23, 25, 27, 29 and 31;
- (e) a polynucleotide having at least 50% sequence identity to SEQ ID NOS: 23, 25, 27, 29 and 31, wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0;
- (f) a polynucleotide comprising at least 25 nucleotides in length which hybridizes under low stringency conditions to a polynucleotide having the sequence set forth in SEQ ID NOS: 23, 25, 27, 29 and 31, wherein the conditions include hybridization with a buffer solution of 30 % formamide, 1 M NaCl, 1% SDS at 37°C for 4-12 hours and a wash in 2X SSC at 50°C;
- (g) a polynucleotide comprising the sequence set forth in SEQ ID NOS: 23, 25, 27, 29 and 31;
- (h) conservatively modified variants of SEQ ID NO 23, 25, 27, 29 and 31;
or
- (i) a polynucleotide complementary to a polynucleotide of (a) through (h).

52. (new) A food or feed comprising the seed of claim 1.

53. (new) The food or feed of claim 52 wherein the food or feed comprises the processed parts of the seed.

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54. (new) The plant CI-2 polypeptide of claim 9, wherein the polypeptide comprises a plant CI-2 derived polypeptide of greater than 50 amino acids in length and is altered to have 8-15 mole % methionine.

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55. (new) The plant CI-2 polypeptide of claim 9, wherein the polypeptide comprising a plant CI-2 derived polypeptide of greater than 50 amino acids in length and is altered to have 13-25 mole % threonine.

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56. (new) The polypeptide of claim 10 wherein the non-native essential amino acid residues comprise lysine and the positions correspond to the positions in SEQ. Id. No. 2 selected from 1, 8, 11, 17, 19, 34, 41, 56, 59, 62, 65, 67, 73 or combinations thereof.

57. (new) Food or feed comprising the polypeptide of claim 30.

58. (new) Food or feed comprising the polypeptide of claim 31.

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59. (new) A CI-2 derived polypeptide of greater than 50 amino acids in length and comprising about 15% or more lysine based on the total number of amino acid residues.

60. (new) The CI-2 derived polypeptide of Claim 59, further comprising about 20% or more lysine based on the total number of amino acid residues.

61.(new) A polypeptide selected from the group consisting of:

- (a) a polypeptide comprising Sequence ID NOS. 6, 8, 10, 12, 14, 16, 18 or 20; and
- (b) a polypeptide comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 modified to contain an essential amino acid at 4 to about 34 positions in a range corresponding to Sequence ID No. 2 positions 19-53 and 63-83.

62.(new) A polypeptide comprising a conservatively modified or conservatively substituted variant of the polypeptide of claim 61, with the proviso that the polypeptide is not a wild type CI-2 polypeptide.

~~63.(new) The polypeptide of claim 61, wherein the essential amino acid is isoleucine, lysine, tryptophan, methionine, threonine, or mixtures thereof.~~

~~64.(new) The polypeptide of claim 61, wherein the essential amino acid is lysine.~~

~~65.(new) The polypeptide of claim 61, further comprising an amino terminal extension.~~

66.(new) The polypeptide of claim 65, wherein the amino terminal extension comprises a start methionine.

67.(new) The polypeptide of claim 65, wherein the amino terminal extension comprises essential amino acids.

68.(new) The polypeptide of claim 61, further comprising at least one non-native cysteine.

- 69.(new) The polypeptide of claim 68, wherein the non-native cysteine is at one or more positions corresponding to Sequence ID No. 2 positions 23, 81, 22, 82, 53 or 70.
- 70.(new) The polypeptide of claim 61, further comprising at least two non-native cysteines.
- 71.(new) The polypeptide of claim 70, wherein the non-native cysteines are at positions (T22C, V82C) or (E23C, R81C).
- 72.(new) A polypeptide of at least 23 amino acids in length comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, modified to contain at least one non-native disulfide bond and at least 4 to about 34 non-native essential amino acids in positions corresponding to Sequence ID No. 2 positions 19-83.
- 73.(new) The polypeptide of claim 72 wherein the non-native essential amino acids are lysine.
- 74.(new) A polypeptide comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, modified to contain a non-native essential amino acid at 6 to about 34 positions in a range corresponding to Sequence ID No. 2 positions 19-53 and 63-83.
- 75.(new) The polypeptide of claim 74 wherein the non-native essential amino acids are lysine.
- 76.(new) A polypeptide of at least 23 amino acids in length comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein the sequence is

modified to contain at least 11 non-native essential amino acids in positions corresponding to Sequence ID No. 2 positions 19-83.

- 77.(new) The polypeptide of claim 76 wherein the non-native essential amino acids are lysine.
- 78.(new) A polypeptide of at least 23 amino acids in length and having at least 64% identity to a sequence of amino acid residues corresponding to positions 19-83 in Sequence ID No. 2 and comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.
- 79.(new) A polypeptide of at least 23 amino acids in length and having at least 60% identity to a sequence of amino acid residues in Sequence ID No. 6 and comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.
- 80.(new) The polypeptide of claim 79, having at least 74% identity to the sequence of amino acid residues.
- 81.(new) A polypeptide of at least 23 amino acids in length and having at least 57% identity to a sequence of amino acid residues in Sequence ID No. 8, and further comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.
- 82.(new) The polypeptide of claim 81, having at least 67% identity to the sequence of amino acid residues.

83. (new) A polypeptide of at least 23 amino acids in length and having at least 57% identity to a sequence of amino acid residues in Sequence ID No. 10 and comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.
84. (new) The polypeptide of claim 83, having at least 67% identity to a sequence of amino acid residues.
85. (new) The polypeptide of claim 84, wherein the cysteine residue is substituted at one or more positions corresponding to Sequence ID NO. 2 positions 23, 81, 22, 82, 53 or 70.
86. (new) The polypeptide of claim 85, wherein the disulfide bond is between positions corresponding to Sequence ID No. 2 positions (22 and 82) or (23 and 81).
87. (new) A polypeptide homologous to CI-2, wherein said polypeptide is at least 20 amino acids in length and is modified to have at least seven non-native essential amino acid residues and comprising a non-native disulfide bond.
88. (new) The homologous polypeptide of claim 87, wherein said homologous polypeptide is a sequence designated by genbank accession number A01293 (*Hordeum vulgare*), Y08625 (*Hordeum vulgare*), S37493 (*Zea mays*), A21463 (*Vicia faba*), S55591 (*Cucurbita maxima*), S12897 (*Cucurbita maxima*), JC2380 (*Canavalia lineata*), JX0089 (*Vigna angularis*), S33547 (*Nicotiana tabacum*), A56555 (*Nicotiana glauca*), Z46949 (*Sambucus nigra*), JC2508 (*Momordica charantia*), S12897 (*Cucurbita maxima*), A01291 (*Solanum tuberosum*), U30861 (*Solanum tuberosum*), A39547 (*Lycopersicon peruvianum*), A32067

(*Lycopersicon esculentum*), A24048 (*Lycopersicon esculentum*), S40496
(*Amaranthus caudatus*) or AC005770 (*Arabidopsis thaliana*).

89.(new) The homologous polypeptide of claim 87, wherein said homologous polypeptide is any one of Sequence ID No. 24, 26, 28, 30 or 32.

90.(new) Seed comprising the polypeptide of claim 87.

91.(new) A plant grown from the seed of claim 90.

92.(new) Food or feed comprising the seed of claim 90.

93.(new) A method for increasing the essential amino acid content in a polypeptide, comprising:

- (a) stably engineering a plant cell to express a polypeptide of at least 23 amino acids in length and having at least seven non-native essential amino acid residues and comprising a non-native disulfide bond; and
- (b) growing the plant cell for a time sufficient to produce the polypeptide in the plant cell.

94.(new) The method of claim 93 wherein the essential amino acid is lysine.

95.(new) The method of claim 93, wherein the plant cell is from corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.

We claim:

1. (once amended) An isolated polypeptideA seed comprising a CI-2-like protein with CI-2 derived polypeptide, which CI-2 derived polypeptide is modified to comprise a non-native essential amino acid residue in more than about 11% to less than about 80% of the amino acid residues.
2. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide of Claim 1 further comprisingfurther comprises post-translational protein modification.
3. The polypeptide of Claim 1 further comprising from one to 5 disulfide bonds.
4. 3. (once amended) The polypeptideseed of Claim 1 wherein the CI-2 derived polypeptide further comprises from one to five disulfide bonds.
4. (once amended) proteinThe seed of Claim 1 wherein the CI-2 derived polypeptide exhibits a free energy of unfolding of more than about 3.5 to less than about 15 Kilocalories per mole.
5. (once amended) The polypeptideseed of Claim 1 wherein the CI-2 derived polypeptide is proteolytically stable, as demonstrated by detection of the intact polypeptide based upon detection by SDS-PAGE analysis, following a 30 minute incubation at 37°C in 100mM Tris-HCl, 50mMNaCl, 1mM CaCl_2 pH 8 , with a 10:1 (weight to weight ratio) of polypeptide:protease, with the protease being either chymotrypsin or trypsin.
6. (once amended) The seed of Claim 1 wherein the CI-2 derived polypeptide of Claim 1 further comprisingfurther comprises an amino-terminal extension.
7. (once amended) The polypeptideseed of Claim 1 wherein the proteinCI-2 derived polypeptide exhibits a modified protease inhibitory activity.
8. (once amended) The polypeptideseed of Claim 1 wherein the non-native essential amino acid residues comprise isoleucine, lysine, threonine, tryptophan, methionine or combinations thereof.
9. (once amended) An isolated polypeptide comprising a plant CI-2-like polypeptideA plant CI-2 derived polypeptide of greater than 50 amino acids in length and altered to have the following composition: 15-35 mole % lysine, 5-158-

15 mole % methionine, 6-25~~13-25~~ mole % threonine, ~~4-9 mole % tryptophan~~6-12 mole % tryptophan, 12-20 mole % isoleucine or combinations thereof.

10. (once amended) An isolated polypeptide comprising Seq. ID. No. 2, or truncated versions thereof, modified to contain 7 or more non-native essential amino acid residues at positions corresponding to the positions in Sequence ID. No. 2 selected from 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81, 82, or combinations thereof.
11. (once amended) The polypeptide of Claim 10 wherein the essential amino acid residues comprise isoleucine, lysine, threonine, tryptophan, methionine, or combinations or conservative substitutions thereof.
12. (no change) The polypeptide of Claim 10 wherein the protein exhibits reduced inhibitory activity against chymotrypsin, subtilisin or elastase.
13. (no change) The protein of claim 10 wherein the polypeptide comprises one or more of the following modifications: V32T; E45T; D64T; D74T; or A77T.
14. (no change) The protein of claim 10 further comprising one of the following modifications: [T22C, V82C], [E23C, R81C] or [V53C, V70C].
15. (no change) The polypeptide of Claim 10 further comprising an amino-terminal extension.
16. (no change) The protein according to claim 15 wherein the amino terminal extension comprises a nutritionally-enhancing polypeptide.
- ~~18-17.~~ (once amended) The polypeptide of Claim 15 wherein the amino-terminal extension is a start signal, a transit sequence, a transit peptide, a signal peptide, a fusion protein, a cleavable peptide, a ~~GI-2-like~~CI-2 like polypeptide or an uncleaved peptide.
18. (once amended) The polypeptide of Claim 15 wherein the ~~GI-2-derived~~ polypeptide comprises at least 1 to about 18 additional residues corresponding to amino acid residues 1 to ~~amino acid residue 17~~18 of Seq. ID No. 2 or 12.

19. (once amended) ~~An isolated~~ A CI-2 derived polypeptide ~~comprising a CI-2 derived~~ protein comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:
- H18A, I, L, V or M; N19K or T; L20M I, or V; E23T or K ; S31T or K; E34K or T; V38M I, or L; L40M I, or V; Q41K or T; Q47K or T; I49M I, L, or V; I56K or T; M59G; R62K or T; I63M, L, or V; R65K or T; R67K or T; F69W; L73K or T; A75K or T; Q78K or T; V79T or K; ~~or R81K~~ and R81K or T.
20. (no change) The polypeptide according to claim 19 wherein the modifications comprise one or more of the following modifications: [E23C and R81C] or [T22C and V82C] or [V53C and V70C].
21. (once amended) ~~An isolated~~ A CI-2 derived polypeptide ~~comprising a CI-2 derived~~ protein comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:
- H18A or M; N19K; L20M; T22C; E23T or C; S31T; E34K; V38M; L40M; Q41K; Q47K; I49M; I56K; M59G; R62K; I63M; R65K; R67K; F69W; L73K; A75K; Q78K; V79T; R81K or C; ~~or~~ and V82C.
22. (once amended) The CI-2 derived polypeptide of claim 21, further comprising substituting a tryptophan at position 61 ~~and a glycine at position 59~~.
23. (no change) The polypeptide of claim 22, further comprising threonine at one or more of positions 32, 45, 53, 64 or 70.
24. (no change) The polypeptide according to claim 22 wherein the modifications comprise one or more of the following modifications: [E23C and R81C] or [T22C and V82C] or [V53C and V70C].
25. (no change) The polypeptide according to claim 22 further comprising an insert in the active site loop region that is enriched in essential amino acids for the purpose of nutritional enhancement.
26. ~~An isolated polypeptide comprising a CI-2 derived protein comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from:~~

~~{one or more of S1 or S2 or V3 or E4 or K5 or K6 or P7 or E8 or G9 or V10 or N11 or T12 or G13 or A14 or G15 or D16 deleted};~~

~~S1K; E8K; N11K; [R17K or M]; [H18A or M]; N19K; L20M; T22 C; [E23T or C]; S31T; V32T; E34K; V38M; L40M; Q41K; E45T; Q47K; I49M; V53C; [[[I56K] and [T58A, or G] and [M59K or G] and [E60A or H] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [R62K]]];~~

~~I63M; D64T; R65K; R67K; F69W; V70C; L73K; D74T; N75K; A77T; Q78K; V79T; [R81K or C]; or V82 C.~~

27. ~~An isolated polypeptide comprising a CI-2 derived protein comprising modifications corresponding to positions in Seq. ID No. 2 selected from: [[[I56K] and [T58A, or G] and [M59K or G] and [E60A or H] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [R62K]]]; and two or more of the following modifications: {one or more of S1 or S2 or V3 or E4 or K5 or K6 or P7 or E8 or G9 or V10 or N11 or T12 or G13 or A14 or G15 or D16 deleted};~~

26. S1K; E8K; N11K; R17K or M; [H18A or M]; N19K; L20M; T22 C; [E23T or C]; S31T; V32T; E34K; V38M; L40M; Q41K; E45T; Q47K; I49M; V53C; I63M; D64T; R65K; R67K; F69W; V70C; L73K; D74T; N75K; A77T; Q78K; V79T; [R81K or C]; or V82 C. (Deleted)

27. (Deleted)

~~An isolated polypeptide of Sequence ID. No. 2 comprising a protein(once amended)~~
A CI-2 derived polypeptide with three or more non-native essential amino acids at positions selected from the group consisting of 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 32, 34, 38, 40, 41, 45, 47, 49, 56, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 73, 74, 75, 76, 77, 78, 79, 81, 82, or 81 and 82; and including conservatively modified and conservatively substituted variants thereof; and excluding V, P, W, S, E and R at position 56; S, K, R, P, E, V, Y, W, and A at position 58; R, Y, P, W, E, V, S,

K, and A at position 59; Q, S, T, I, P, and K at position 60; V, E, R, P, and W at position 61 and E, Q, N, V, F, and Y position 62—~~and conservatively modified and conservatively substituted variants thereof.~~

28. 62.

29. (once amended) ~~An isolated polypeptide comprising Seq. ID No. 6, 8, 10, 12, 14, 16, 18, 20 or conservatively modified or conservatively substituted variants thereof.~~

30. (once amended) ~~An isolated polypeptide comprising at least 23 contiguous amino acids of SEQ. ID Nos. 6, 8, 10, 12, 14, 16, 18, 20 or 20.~~

31. (once amended) ~~An isolated polypeptide comprising at least 23 contiguous amino acids with more than 79% sequence identity, to the polypeptide of Seq. ID No. 20, wherein the % sequence identity is based on the 23 contiguous amino acids sequence and is determined by GAP analysis using Gap Weight of 12 and Length Weight of 4.~~

32. (once amended) ~~An isolated~~ A CI-2 derived polypeptide that is immunologically reactive with antibodies against the protein of Seq. ID No. 20 and not SEQ ID No. 2.

33. (no change, non-elected claim) An isolated nucleic acid comprising:

- (a) a polynucleotide encoding the protein of claim 1;
- (b) a polynucleotide that encodes a polypeptide of SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, or 20;
- (c) a polynucleotide amplified from a plant nucleic acid library using the primers of SEQ ID NOS: 21 and 22;
- (d) a polynucleotide comprising at least 20 contiguous bases of SEQ ID NOS: 5, 7, 9, 11, 13, 15, 17 or 19;
- (e) a polynucleotide encoding a plant CI-2-derived polypeptide having 15% more essential amino acids than SEQ ID NO 2;

- (f) a polynucleotide having at least 73% sequence identity to SEQ ID NO: 19, wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0;
 - (g) a polynucleotide comprising at least 25 nucleotides in length which hybridizes under low stringency conditions to a polynucleotide having the sequence set forth in SEQ ID NOs: 19, wherein the conditions include hybridization with a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37°C for 24 hours and a wash in 2X SSC at 50°C, 3x for 15 minutes;
 - (h) a polynucleotide comprising the sequence set forth in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17 or 19;
 - (i) conservatively modified variants of SEQ ID NO : 5, 7, 9, 11, 13, 15, 17 or 19; or
 - (j) a polynucleotide complementary to a polynucleotide of (a) through (i).
34. (no change, non-elected claim) The isolated nucleic acid of claim 33 wherein the polynucleotide is a plant polynucleotide.
35. (no change, non-elected claim) A vector comprising at least one nucleic acid of claim 33.
36. (no change, non-elected claim) An expression cassette comprising at least one nucleic acid of claim 33 operably linked to a promoter, wherein the nucleic acid is in sense or antisense orientation.
37. (no change, non-elected claim) A host cell into which is introduced at least one expression cassette of claim 36.
38. (no change, non-elected claim) The host cell of claim 37 that is a plant cell.
39. (no change, non-elected claim) A transgenic plant comprising at least one expression cassette of claim 36.
40. (no change, non-elected claim) The transgenic plant of claim 39, wherein the plant is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.
41. (no change, non-elected claim) A seed from the transgenic plant of claim 40.

42. (no change, non-elected claim) The seed of claim 41, wherein the seed is from corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley lupin or millet.
43. (no change, non-elected claim) A ribonucleic acid sequence encoding a polypeptide of claim 10.
44. (once amended, non-elected claim) A method for increasing the essential amino acid content in a polypeptide in a plant, comprising:
- (a) stably transforming a plant cell with the polynucleotide encoding the polypeptide of claim 10, operably linked to a promoter, wherein the polynucleotide is in sense orientation;
 - (b) growing the plant cell under plant growing conditions to produce a regenerated plant; and
 - (c) ~~expressing the polypeptide~~ growing plants for a time sufficient to ~~produce~~ express the polypeptide encoded by the polynucleotide of (a) in the plant.
45. (no change, non-elected claim) The method of claim 44, wherein the plant cell is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.
46. (once amended, non-elected claim) A method of increasing expression levels of a ~~protein~~ polypeptide in a transgenic plant cell comprising:
- engineering a nucleotide sequence encoding the protein of interest ~~polypeptide~~ to contain a non-native disulfide bond to increase the in-vitro proteolytic or thermodynamic stability of the ~~protein~~ polypeptide; introducing at least 1 copy into a plant cell; and
- of the polypeptide into the transgenic plant cell; and growing the transgenic plant cell expressing the protein.
47. ~~A method of increasing nutritional value of feed comprising substituting more than 11% to less than 75% of the amino acids residues of a protein with essential amino acids and modifying the protein to increase the stability of the in-vivo~~

~~expressed polypeptide for a time sufficient to express the polypeptide.~~

47. (once amended, non-elected claim but w/ request to examine) A method of increasing the nutritional value of a feed or food source comprising producing the seed of Claim 1 and incorporating such seed into the feed or food source.
48. (deleted)
49. (once amended, non-elected claim but w/ request to examine) The method of Claim 47 wherein the ~~modifying stability is from one or more~~ seed of Claim 1 further comprises one or more non-native disulfide bonds.
50. (once amended, non-elected claim but w/ request to examine) A method of increasing the nutritional value of a protein by altering a CI-2 homologue to enhance its nutritional value by altering two or more of the amino acid residues to the at positions in Sequence ID No. 2 selected from the group consisting of: 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81, 82, or combinations thereof. 81 and 82 to have an essential amino acid, wherein the positions are determined based on homology to the amino acid positions in Sequence ID No. 2.
51. (deleted) An isolated nucleic acid comprising:
- (a) a polynucleotide of Seq ID Nos 23, 25, 27, 29 and 31;
 - (b) ~~a~~ polynucleotide that encodes a polypeptide of SEQ ID NOS: 24, 26, 28, 30, 32;
 - (c) a polynucleotide amplified from a Zea mays nucleic acid library using the primers of SEQ ID NOS: 21 and 22;
 - (d) a polynucleotide comprising at least 20 contiguous bases of SEQ ID NOS: 23, 25, 27, 29 and 31;
 - (e) a polynucleotide having at least 50% sequence identity to SEQ ID NOS: 23, 25, 27, 29 and 31, wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0;
 - (f) a polynucleotide comprising at least 25 nucleotides in length which hybridizes under low stringency conditions to a polynucleotide having

the sequence set forth in SEQ ID NOs: 23, 25, 27, 29 and 31, wherein the conditions include hybridization with a buffer solution of 30 % formamide, 1 M NaCl, 1% SDS at 37°C for 4-12 hours and a wash in 2X SSC at 50°C;

- (g) a polynucleotide comprising the sequence set forth in SEQ ID NOs: 23, 25, 27, 29 and 31;
- (h) conservatively modified variants of SEQ ID NO 23, 25, 27, 29 and 31;
or
- (i) a polynucleotide complementary to a polynucleotide of (a) through (h).

52. (new) A food or feed comprising the seed of claim 1.

53. (new) The food or feed of claim 52 wherein the food or feed comprises the processed parts of the seed.

54. (new) The plant CI-2 polypeptide of claim 9, wherein the polypeptide comprises a plant CI-2 derived polypeptide of greater than 50 amino acids in length and is altered to have 8-15 mole % methionine.

55. (new) The plant CI-2 polypeptide of claim 9, wherein the polypeptide comprising a plant CI-2 derived polypeptide of greater than 50 amino acids in length and is altered to have 13-25 mole % threonine.

56. (new) The polypeptide of claim 10 wherein the non-native essential amino acid residues comprise lysine and the positions correspond to the positions in SEQ. Id. No. 2 selected from 1, 8, 11, 17, 19, 34, 41, 56, 59, 62, 65, 67, 73 or combinations thereof.

57. (new) Food or feed comprising the polypeptide of claim 30.

58. (new) Food or feed comprising the polypeptide of claim 31.

59. (new) A CI-2 derived polypeptide of greater than 50 amino acids in length and comprising about 15% or more lysine based on the total number of amino acid residues.

60. (new) The CI-2 derived polypeptide of Claim 59, further comprising about 20% or more lysine based on the total number of amino acid residues.

61. (new) A polypeptide selected from the group consisting of:

- (a) a polypeptide comprising Sequence ID NOS. 6, 8, 10, 12, 14, 16, 18 or 20; and
- (b) a polypeptide comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 modified to contain an essential amino acid at 4 to about 34 positions in a range corresponding to Sequence ID No. 2 positions 19-53 and 63-83.

62. (new) A polypeptide comprising a conservatively modified or conservatively substituted variant of the polypeptide of claim 61, with the proviso that the polypeptide is not a wild type CI-2 polypeptide.

63. (new) The polypeptide of claim 61, wherein the essential amino acid is isoleucine, lysine, tryptophan, methionine, threonine, or mixtures thereof.

64. (new) The polypeptide of claim 61, wherein the essential amino acid is lysine.

65. (new) The polypeptide of claim 61, further comprising an amino terminal extension.
66. (new) The polypeptide of claim 65, wherein the amino terminal extension comprises a start methionine.
67. (new) The polypeptide of claim 65, wherein the amino terminal extension comprises essential amino acids.
68. (new) The polypeptide of claim 61, further comprising at least one non-native cysteine.
69. (new) The polypeptide of claim 68, wherein the non-native cysteine is at one or more positions corresponding to Sequence ID No. 2 positions 23, 81, 22, 82, 53 or 70.
70. (new) The polypeptide of claim 61, further comprising at least two non-native cysteines.
71. (new) The polypeptide of claim 70, wherein the non-native cysteines are at positions (T22C, V82C) or (E23C, R81C).
72. (new) A polypeptide of at least 23 amino acids in length comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, modified to contain at least one non-native disulfide bond and at least 4 to about 34 non-native essential amino acids in positions corresponding to Sequence ID No. 2 positions 19-83.
73. (new) The polypeptide of claim 72 wherein the non-native essential amino acids are lysine.

74. (new) A polypeptide comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, modified to contain a non-native essential amino acid at 6 to about 34 positions in a range corresponding to Sequence ID No. 2 positions 19-53 and 63-83.

75. (new) The polypeptide of claim 74 wherein the non-native essential amino acids are lysine.

76. (new) A polypeptide of at least 23 amino acids in length comprising any one of Sequence ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein the sequence is modified to contain at least 11 non-native essential amino acids in positions corresponding to Sequence ID No. 2 positions 19-83.

77. (new) The polypeptide of claim 76 wherein the non-native essential amino acids are lysine.

78. (new) A polypeptide of at least 23 amino acids in length and having at least 64% identity to a sequence of amino acid residues corresponding to positions 19-83 in Sequence ID No. 2 and comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.

79. (new) A polypeptide of at least 23 amino acids in length and having at least 60% identity to a sequence of amino acid residues in Sequence ID No. 6 and comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.

80. (new) The polypeptide of claim 79, having at least 74% identity to the sequence of amino acid residues.
81. (new) A polypeptide of at least 23 amino acids in length and having at least 57% identity to a sequence of amino acid residues in Sequence ID No. 8, and further comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.
82. (new) The polypeptide of claim 81, having at least 67% identity to the sequence of amino acid residues.
83. (new) A polypeptide of at least 23 amino acids in length and having at least 57% identity to a sequence of amino acid residues in Sequence ID No. 10 and comprising a non-native disulfide bond with at least one cysteine residue in at least one position corresponding to positions 19-83 in Sequence ID No. 2.
84. (new) The polypeptide of claim 83, having at least 67% identity to a sequence of amino acid residues.
85. (new) The polypeptide of claim 84, wherein the cysteine residue is substituted at one or more positions corresponding to Sequence ID NO. 2 positions 23, 81, 22, 82, 53 or 70.
86. (new) The polypeptide of claim 85, wherein the disulfide bond is between positions corresponding to Sequence ID No. 2 positions (22 and 82) or (23 and 81).

87. (new) A polypeptide homologous to CI-2, wherein said polypeptide is at least 20 amino acids in length and is modified to have at least seven non-native essential amino acid residues and comprising a non-native disulfide bond.

88. (new) The homologous polypeptide of claim 87, wherein said homologous polypeptide is a sequence designated by genbank accession number A01293 (Hordeum vulgare), Y08625 (Hordeum vulgare), S37493 (Zea mays), A21463 (Vicia faba), S55591 (Cucurbita maxima), S12897 (Cucurbita maxima), JC2380 (Canavalia lineata), JX0089 (Vigna angularis), S33547 (Nicotiana tabacum), A56555 (Nicotiana glauca), Z46949 (Sambucus nigra), JC2508 (Momordica charantia), S12897 (Cucurbita maxima), A01291 (Solanum tuberosum), U30861 (Solanum tuberosum), A39547 (Lycopersicon peruvianum), A32067 (Lycopersicon esculentum), A24048 (Lycopersicon esculentum), S40496 (Amaranthus caudatus) or AC005770 (Arabidopsis thaliana).

89. (new) The homologous polypeptide of claim 87, wherein said homologous polypeptide is any one of Sequence ID No. 24, 26, 28, 30 or 32.

90. (new) Seed comprising the polypeptide of claim 87.

91. (new) A plant grown from the seed of claim 90.

92. (new) Food or feed comprising the seed of claim 90.

93. (new) A method for increasing the essential amino acid content in a polypeptide, comprising:

(a) stably engineering a plant cell to express a polypeptide of at least 23 amino acids in length and having at least seven non-native essential amino acid residues and comprising a non-native disulfide bond; and

(b) growing the plant cell for a time sufficient to produce the polypeptide in the plant cell.

94. (new) The method of claim 93 wherein the essential amino acid is lysine.

95. (new) The method of claim 93, wherein the plant cell is from corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.